Potassium chloride deters *Lygus hesperus* feeding behavior

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Abstract

A series of bioassays were conducted to determine the response of adult western tarnished plant bugs, Lygus hesperus Knight (Heteroptera: Miridae), to artificial diets containing potassium chloride (KCl). We first examined the feeding behavior of *L. hesperus* by direct observation in a no-choice diet feeding arena. We observed a total of 22 Lygus feeding events lasting an average of 411 ± 64 s on the control artificial diet and only three feeding events lasting an average of 11 ± 3 s on the KCl-treated diet. We then conducted several multiple diet choice bioassays to determine the feeding response of *L. hesperus* when exposed simultaneously to five artificial diet treatments containing different amounts of KCl. For the first bioassay, we used standard clear parafilm diet packets and for the second bioassay we used dark green parafilm diet packets to hold the various diet treatments. Regardless of the diet packet color, L. hesperus overwhelmingly selected the 0% KCl diet treatment over diets containing 3, 6, 9, or 12% KCl. The third and fourth multiple diet choice bioassays were identical to the first bioassay, except that concentrations of the KCl-treated diets were reduced. Lygus hesperus consistently selected the control diet over all diets containing more than 0.5% KCl. However, when the concentration of KCl in the diet was reduced to ≤0.4%, there were no significant differences in feeding activity exhibited by L. hesperus. Finally, to determine if the addition of KCl to the diet influenced their upwind response, we examined the responses of L. hesperus that were simultaneously exposed to a control artificial diet and a diet containing 12% KCl in a Y-tube olfactometer bioassay. Of the 95 adults tested, 47 selected the arm containing the normal diet and 48 selected the arm containing KCl-treated diet, indicating that dietary constituents did not preferentially attract or repel L. hesperus. The results from these studies strongly suggest that KCl negatively affects L. hesperus feeding behavior by functioning as a strong gustatory deterrent when concentrations exceed 0.5%. Visual and volatile cues appeared to have no role in mediating orientation or feeding behavior under these test conditions.

Introduction

The western tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae), and other *Lygus* species are pests of many crops, including alfalfa, cotton, beans, peas, safflower, palm and stone fruits, and strawberries. These bugs feed primarily on developing reproductive structures, such as buds, blooms, and seeds or on growing plant terminals (Jackson et al., 1995). To date, the primary method used to control *Lygus* spp. has been through the use of broad-spectrum insecticides (Nordlund, 2000; Scott & Snodgrass, 2000). Unfortunately, the use of many of these insecticides has caused outbreaks of secondary pests

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(Falcon et al., 1968; Eveleens et al., 1973; Walker & Smith, 1996), destruction of natural enemy complexes (Naranjo et al., 2004; Hagler & Naranjo, 2005), and development of insecticide resistance (Xu & Brindley, 1993; Snodgrass, 1994, 1996; Miller, 1996; Zhu et al., 2004).

Problems associated with using insecticides for *L. hesperus* control have prompted researchers to investigate alternative pest control strategies (e.g., biological control, cultural control, and mechanical control; see Nordlund & Hardee, 2000 for a review). One such control tactic that has been studied extensively, but has never come to fruition, is the use of a *L. hesperus*-specific sex pheromone that could be used to lure *L. hesperus* into a trap or disrupt their mating behavior (Hedin et al., 1985; Chinta et al., 1994; McLaughlin, 1998; Millar et al., 2000; Ho & Millar, 2002; Wardle et al.,

2003; Blackmer et al., 2004; Innocenzi et al., 2004). An alternative behavioral control management tactic that has not been thoroughly examined is the identification of substances that might repel *L. hesperus* from a host plant or deter it from feeding (Hatfield et al., 1982, 1983). Many substances have been shown to inhibit insect feeding behavior (Baker, 1977; Baker & Baker, 1983; Inouye & Waller, 1984; Hagler & Buchmann, 1993; Frances et al., 2004). One such substance is potassium. For example, honey bee, *Apis mellifera* L., foraging activity was found to be inversely proportional to the concentration of potassium in onion nectar (Waller, 1972; Hagler, 1990). More recently, high concentrations of potassium in leaf tissue were found to be negatively correlated with insect population densities (Facknath & Lalljee, 2005; Myers & Gratton, 2006).

This study was designed to determine if potassium chloride (KCl) inhibits *L. hesperus* feeding activity. Specifically, we conducted several gustatory feeding bioassays (i.e., a no-choice diet bioassay and multiple-choice diet bioassays) to determine if the addition of KCl to *L. hesperus* artificial diet affected its feeding behavior. We then conducted a Y-tube olfactory bioassay to determine if *L. hesperus* responded to volatile dietary cues in our parafilm diet packets.

Materials and methods

Test insects

All *L. hesperus* used in the experiments described below were obtained from a laboratory-reared colony located at the Western Cotton Research Laboratory (Phoenix, AZ, USA). The colony was continuously fed a well-established artificial diet described by Debolt (1982). The rearing room was maintained at 30 °C, 30% r.h., and L14:D10 photoperiod. All *L. hesperus* used in the bioassays described below were 2- to 3-day-old adults.

No-choice diet bioassay

Diet preparation. A standard batch of artificial diet was prepared as described by Debolt (1982) and then divided into two 150-ml aliquots. Each aliquot was then treated with either 0 (standard control diet) or 12.0% KCl (potassium chloride, ACS Reagent, 99.0%; Sigma-Aldrich, St. Louis, MO, USA). The diet treatments were stirred continuously for 5 min, poured into separate 4.5 × 4.5-cm green Florist Parafilm stem wrap (American National Can™, Neenah, WI, USA) diet packets, and the parafilm was heat sealed to prevent leakage.

Feeding arena. Two hours before each behavioral observation, a single adult *L. hesperus* was removed from the laboratory colony and placed in a 14.5-cm Petri dish. After 2 h, an

Table 1 Descriptions of the behavioral events observed for *Lygus hesperus* adults exposed to a control artificial diet packet or an artificial diet packet containing 12% potassium chloride

Observed behavior	Description of behavior
Walking	Lygus moving forward across the feeding arena
Resting	Lygus standing motionless
Grooming	Lygus making rapid movements with its fore and hind legs across its body surface and antennae
Probing	<i>Lygus</i> inserting its mouthparts into the diet packet, but not feeding for any length of time (e.g., <5 s)
Feeding	<i>Lygus</i> inserting its mouthparts into the diet packet and feeding for an extended amount of time (e.g., >5 s)

individual was introduced into a 5.5-cm Petri dish arena containing a green diet packet with either a standard control (0% KCl) artificial diet or a diet containing 12% KCl. Each individual was continuously monitored in the feeding arena by direct focal observation for 15 min. Previous observations of L. hesperus behavior in a feeding arena revealed several distinct behaviors (Hagler et al., 2004). Subsequently, a behavioral ethogram (Lehner, 1979) was developed and its components were programed into The Observer®, a software program designed specifically for animal behavior research (Noldus Information Systems, version 3.0, Wageningen, The Netherlands). Descriptions of L. hesperus behaviors that were recorded during this study are given in Table 1. After each 15-min observation period, another randomly selected L. hesperus was observed in a new feeding arena. A total of 20 individuals (10 males and 10 females) were observed feeding on the control diet and the diet containing 12% KCl diet, respectively.

Data analysis. There were no differences detected in the feeding behaviors of male and female *L. hesperus* within the two diet treatments. Therefore, the data obtained by males and females were pooled to increase the sample size to 20 individuals per diet treatment. The average time for each *L. hesperus* feeding event on the two diet treatments was compared by a t-test. The timed durations for each individual grooming, probing, resting, and walking event did not conform to the assumption of normality, so a Mann–Whitney rank sum test was used to determine significant differences between the two diet treatments (SigmaStat, version 2.03, SPSS Inc., Chicago, IL, USA).

Multiple diet choice bioassay - response to high KCl concentrations *Diet preparation.* A standard batch of artificial diet was prepared and then divided into five 150-ml aliquots. Each

aliquot was then treated with either 0 (standard control diet), 3, 6, 9, or 12% KCl. The diet treatments were stirred continuously for 5 min, poured into separate 10.1 × 7.6-cm clear parafilm (Parafilm Laboratory Film, Menasha, WI, USA) diet packets (30 ml of diet per packet), and the parafilm was heat sealed to prevent leakage.

Test arena. The test arena consisted of 15 diet packets placed equidistant in a 3×5 grid on a 50.8×38.1 cm plastic cafeteria serving tray (Plaza Plastics Corp, Houston, TX, USA). The three rows of the grid were arranged as experimental blocks, each containing a single diet packet representing one of the five KCl diet treatments (each treatment was randomized within each row). Each diet packet was taped to the cafeteria tray around its edges using transparent tape so L. hesperus could not crawl under the packets to feed. Then, 250 L. hesperus (1:1 sex ratio) were added to the arena and the arena was immediately covered with a 53.1 × 40.6 cm glass plate. A Sony digital HandyCam™ (model DCR-TRV 103 NTSC) was placed above the arena and set to continuously record the diet choice of L. hesperus on a VHS tape (Sony Electronics Inc., Willowdale, Ontario, Canada) for 48 h using a Sony Time Lapse VCR (model SVT-S3100). Lygus hesperus was given 1 h to acclimatize to the arena before the recording was initiated. The diet choice exhibited by L. hesperus was determined by fast forwarding the taped recording and pushing the freeze-frame button on the VCR at the beginning of each hour of the 48 h of recording and then counting the number of L. hesperus on each diet packet. The feeding bioassay was conducted under constant light (330 lux), temperature (27 °C), and relative humidity (20%).

Statistical analysis. The 48-h feeding bioassay was repeated three times. The data obtained from the three bioassays were combined, yielding a sample size of 432 observations per treatment (e.g., 3 bioassays × 3 blocks per bioassay × 48, 1 h observations). The cumulative number of L. hesperus feeding on each diet treatment over time was tallied to graphically depict the rapid response (or lack thereof) of L. hesperus to the various diet treatments. Then, the number of L. hesperus feeding on the various diet treatments was analyzed by Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks using SigmaStat because both non-transformed and transformed data were not normally distributed and did not contain equal variances. Tukey's multiple comparison tests were used to identify significant differences in the number of feeding events between the five diet treatments.

Multiple diet choice bioassay - response to color

The addition of increasing concentrations of KCl to the L. hesperus diet caused a distinctive color change (e.g., darker) in the diet, which could be easily detected by human eye through the translucent parafilm diet packets. Therefore, a second multiple diet choice bioassay was conducted that was identical to the one described above with one exception; the standard translucent parafilm diet packets were replaced with solid green Florist Parafilm stem wrap diet packets. The dark green parafilm packets eliminated the possibility of L. hesperus selecting one diet treatment over another by direct visual cues.

Multiple diet choice bioassays - response to reduced KCI concentrations

Two more multiple diet choice bioassay studies were conducted that were similar to the first multiple choice bioassay described above with a few modifications. In the first study, concentrations of KCl were reduced to 0 (control), 0.5, 1.0, 1.5, or 2.0% KCl. In the second study, KCl concentrations were further reduced to 0, 0.1, 0.2, 0.3, or 0.4% KCl. Count data were only collected at the end of 24 and 48 h periods for each bioassay, respectively.

Y-tube olfactometer bioassay - response to artificial diet parafilm packets

Y-tube olfactometer set-up. Preliminary gas chromatographymass spectrometry (GC-MS) analysis of the headspace of punctured (to represent L. hesperus feeding) parafilm packets showed two volatile compounds were released with retention times of 3.95 and 10.0 min, respectively (J Byers, unpubl.). These compounds were not detected in parafilm alone or from unpunctured diet packets. Thus these two volatiles represent potential dietary cues to which L. hesperus might respond. To determine if this factor influenced orientation behaviors of L. hesperus, bioassays were conducted in a 36-cm long glass Y-tube × 40 mm in diameter olfactometer that had a 50° inside angle (see Blackmer et al., 2004 for details). Incoming air was filtered through activated charcoal and humidified with double distilled, deionized water. The filtered air was split between two 2-l holding chambers; one chamber served as a control, holding a diet packet containing normal diet (0% KCl) and the other chamber contained a diet packet containing 12% KCl. The diet packets were similar to those described above with two minor modifications. First, the size of each diet packet was reduced (4.5 × 4.5 cm) to fit into the Y-tube olfactometer; and second, 20 holes were punctured into each diet packet with a #3 insect pin to simulate L. hesperus stylet penetration into the diet packets. From each holding chamber, the air passed into the respective arms of the Y-tube, and then through a series of screens before entering the main tube of the olfactometer. Airflow through the system was maintained at 4.8 l per

min (= 3.8 m per min inside the tube) by an inline flowmeter (Gilmont Instr., Barnant Co., Barrington, IL, USA). A smoke test demonstrated uniform laminar airflow in both arms and throughout the olfactometer. Light-emitting diodes (LED) (Green LED, NSPG520S, Nichia America Corp., Mountville, PA, USA) were used to simulate a visual plant cue. The lights emitted a narrow wavelength in the range of 530 nm, and power was supplied by a universal adapter that provided 6 V direct current. The LEDs were positioned behind the organdy screens at the ends of both arms of the Y-tube. A 60-cm long, widespectrum fluorescent lamp (F20T12-PL/AQ; General Electric, Fairfield, CT, USA) was positioned 22 cm above the arms of the Y-tube. Before each trial, light intensity over each arm was measured with a light meter (ExTech Instr., model 401025, Zefon International, St. Petersburg, FL, USA), and the tube was adjusted until intensity was the same in both arms. Light intensity averaged 690.5 \pm 6.9 lux. The Y-tube arena was surrounded by a $50 \times 70 \times$ 60 cm black fabric enclosure, and the holding chambers containing the treatments were placed outside the enclosure to eliminate external visual cues.

Bioassays. Approximately 30 min before each trial was initiated, 2- to 3-day-old *L. hesperus* were placed into individual holding/release tubes. Each tube was constructed from a 15-cm long, 5-ml plastic pipette from which 0.5 cm of the bulb and 8 cm of the pipette tip was removed. The cut end of the pipette tip was covered with organdy. An adult was placed inside the tube and the end where the bulb tip had been removed was sealed with a cork. Tubes containing bugs were then placed into a separate holding container, so they would not be exposed to potential test odors before their release.

At the beginning of each trial, the cork was removed from the holding/release tube, and the open end was placed at the downwind end of the Y-tube. Each insect was given 5 min to respond to the treatment, and a choice for the left or right arm of the olfactometer was recorded when the insect went 1 cm past the Y-junction. Temperature and r.h. in the olfactometer were maintained at 25.0 \pm 0.7 °C and 20.0 \pm 8.5%, respectively. The Y-tubes were thoroughly cleaned after each trial with soapy water, distilled water, 70% alcohol, and acetone. Each day that a set of trials was conducted, the treatments were rotated between the arms of the Y-tube to eliminate any potential bias. A total of 32 females and 63 males were tested for response to the two diet treatments.

The null hypothesis that each sex showed no preference for either diet treatment (a response equal to 50:50) was analyzed with a χ^2 goodness-of-fit test after correcting for continuity with Yates' correction factor (Zar, 1984).

Results

No-choice diet bioassay

No-choice diet bioassays with individual *L. hesperus* exposed to either the standard control diet or a diet containing 12% KCl revealed that the control diet was highly preferred over the diet containing KCl. Specifically, 17 of the 20 L. hesperus observed for 15 min each on the control diet fed at least once and for an extended period of time. In all, 22 L. hesperus feeding events lasting an average of 411 ± 64 s were observed on the control diet (Figure 1). Conversely, only three feeding events, lasting an average of only 11 ± 3 s were recorded for the 20 L. hesperus observed in the arena containing the KCl-treated diet packet. The other major difference in the behaviors exhibited by L. hesperus between the two diet treatments was the duration of their probing events. Specifically, they probed on the KCl-treated diet more frequently and for a significantly longer period of time than they did on the normal diet (Figure 1). The average duration of L. hesperus grooming, resting, and walking events were not significantly different between the two diet treatments. However, the number of grooming events was almost twice as high for L. hesperus exposed to the KCl-treated diet.

Multiple diet choice bioassay - response to high KCl concentrations Within the first hour of the bioassay, *L. hesperus* showed a distinct preference for the control diet over diets containing

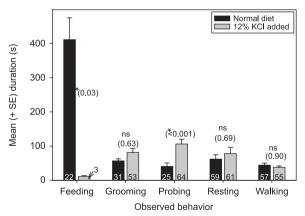
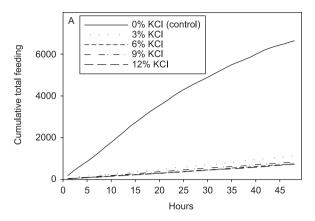


Figure 1 Average (+ SE) duration of each behavioral event recorded for *Lygus hesperus* exposed to either a control artificial diet [0% potassium chloride (KCl)] or a diet containing 12% KCl. Numbers inside the vertical bars represent the frequency (sample size) of each behavior. Significant differences in time between the two diet treatments were determined by t-test for the feeding behavioral event and by the Mann–Whitney rank sum test for the grooming, probing, resting, and walking behavioral events. Numbers in parentheses represent the P-values. The asterisk indicates significant differences between the treatments. ns, non-significant.



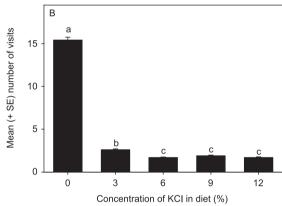


Figure 2 (A) Forty-eight hours cumulative total number of *Lygus hesperus* feeding on clear artificial diet packets containing 0, 3, 6, 9, or 12% potassium chloride (KCl). (B) Overall mean (+ SE) number of *L. hesperus* feeding on clear artificial diet packets containing KCl. Different letters indicate significant differences between the treatment means as determined by Tukey's multiple comparison test (H = 1068.1, d.f. = 4, P<0.001; n = 432 per treatment).

3, 6, 9, or 12% KCl (Figure 2A). On average, 15.4 \pm 0.3 *L. hesperus* were observed feeding on the control diet while <2.6 \pm 0.1 were observed feeding on the KCltreated diets (Figure 2B).

Multiple diet choice bioassay - response to color

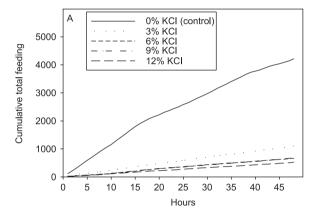
The diet preference exhibited by *L. hesperus* on the dark green diet packets was virtually identical to the results obtained in the first multiple diet choice bioassay with clear parafilm. That is, they quickly and consistently selected the control diet over the KCl-treated diets (Figure 3A,B).

Multiple diet choice bioassays – response to reduced KCl concentrations

The third and fourth multiple-choice bioassays were conducted using artificial diet treatments containing lower concentrations of KCl than in the previous bioassays. The diet choice exhibited by *L. hesperus* in the third bioassay showed that artificial diets containing \geq 0.5% KCl deterred *L. hesperus* feeding activity in a concentration-dependent manner (Figure 4A,B). However, the results yielded from the fourth feeding bioassay revealed no significant differences (data not shown) in feeding activity between any of the treatments with the mean number of *L. hesperus* feeding on the diet packets, ranging from an average of 5.0 ± 0.26) visits on the 0.1% KCl diet to 5.8 ± 0.25) visits on the 0.2% KCl diet (H = 14.6, d.f. = 4, P = 0.06; n = 144).

Y-tube olfactometer - response to artificial diet parafilm packets

The Y-tube olfactometer bioassay was conducted to determine if the two volatiles emitted from the punctured parafilm packets influenced orientation behaviors of *L. hesperus*.



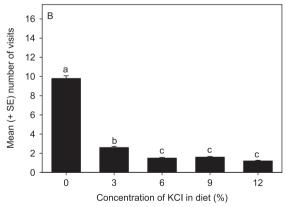
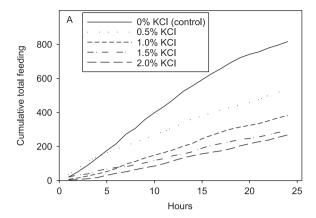


Figure 3 (A) Forty-eight hours cumulative total number of *Lygus hesperus* feeding on dark green artificial diet packets containing 0, 3, 6, 9, or 12% potassium chloride (KCl). (B) Overall mean (+ SE) number of *L. hesperus* feeding on clear artificial diet packets containing KCl. Different letters indicate significant differences between the treatment means as determined by Tukey's multiple comparison test (H = 936.7, d.f. = 4, P<0.001; n = 432 per treatment).



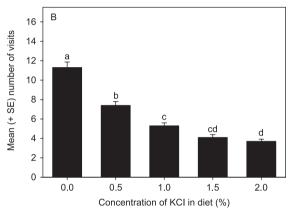


Figure 4 (A) Twenty-four hours cumulative total number of *Lygus hesperus* feeding on clear artificial diet packets containing 0, 0.5, 1, 1.5, or 2% potassium chloride (KCl). (B) Overall mean (+ SE) number of *L. hesperus* feeding on clear artificial diet packets containing KCl. Different letters indicate significant differences between the treatment means as determined by Tukey's multiple comparison test (H = 143.1, d.f. = 4, P < 0.001; n = 72 per treatment).

Both male and female *L. hesperus* bugs showed no preference between the control diet and the KCl-treated diet. When the data for the males and females were combined, 47 *L. hesperus* selected the control diet and 48 selected the 12% KCl diet (Figure 5). These results demonstrate that the feeding responses observed in our previous experiments were not related to volatile cues emitted from the punctured packets.

Discussion

These studies show that KCl is a strong gustatory feeding deterrent for *L. hesperus*. Specifically, *L. hesperus* consistently preferred the control diet over diets containing ≥0.5% KCl, and the potential visual and volatile cues did not influence

their diet choice. The addition of KCl to the artificial diet made it appear darker than the control diet. However, in the subsequent multiple diet choice feeding bioassay, the possibility of *L. hesperus* selecting the control diet over the KCl-treated diet by color was eliminated by presenting the diet treatments in dark green, non-translucent diet packets.

We speculated that the two volatile compounds associated with the punctured diet packets might influence upwind orientation (J Byers, unpubl.). However, the Y-tube bioassay results demonstrated that *L. hesperus* were not responding to the volatiles.

The specific reason why KCl deters L. hesperus is unknown. There is very little information published on the impact of KCl or any other single salt on insect feeding activity. KCl has been shown to alter the metabolism of certain insects that feed on KCl-enriched diets (Bhattacharya & Kaliwal, 2005). However, our study showed that L. hesperus avoided the KCl diets in both the multiple diet choice and no-choice diet bioassays. Strong & Kruitwagen (1970) tested the preference of *L. hesperus* to dozens of potential artificial diets using a multitude of two-way diet choice tests. A component of the control diet (i.e., the diet they perceived as the best potential diet) they tested against numerous other diet mixes included a complex salt mixture that contained a small amount of KCl. Specifically, KCl constituted only 3% of the total salt mixture and 0.02% of the total diet mixture (e.g., all the salts, amino acids, vitamins, lipids, and other ingredients combined). They concluded that altering the amount of salt presented to L. hesperus did not have any noticeable negative affect on L. hesperus feeding activity until it was increased six times over the amount of the control diet. Whether the decrease in L. hesperus feeding on the salt-enriched diet

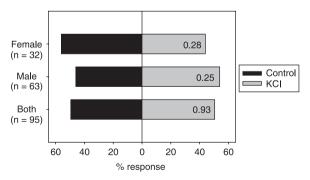


Figure 5 Upwind walking frequency response of adult *Lygus hesperus* to artificial diet packets containing 0 and 12% KCl. Numbers in parentheses represent sample size; χ^2 -values for each paired comparison are given in the shaded regions of the figure. All χ^2 tests were non-significant (P<0.50).

was due to the increased concentration of KCl alone or to the combination of all the salts is unknown.

To date, most of the research on salt chemoreception by insects has been conducted on flies, cockroaches, butterflies, and moths (see Diether, 1977; Bernays & Chapman, 2001; Merivee et al., 2004 for reviews). High concentrations of potassium in the nectar of certain onion varieties deter honey bees, which Waller (1972) suggested might be due to a bitter and unfavorable taste. Salt taste reception is highly variable between various insect groups and deserves further investigation on L. hesperus (Schnuch, 1996; Bernays & Chapman, 2001; Hansen-Delkeskamp, 2001).

The effects of KCl and other salts (primarily NaCl) on insect feeding and lifespan suggest that salts might be used for cultural insect control. For example, longhorn pine beetle Hylotrupetes bajulus (L.) survival was significantly reduced when it was exposed to pine (Pinus sylvestris L.) blocks impregnated with either KCl or NaCl (Hertel, 1997). Addition of table salt (NaCl) to lower doses of certain topically applied insecticides synergized the activity of the insecticides for stink bug control (Corso & Gazzoni, 1998; Khan et al., 2002). The application of potassium-based fertilizers has been shown to increase the total potassium in certain plant tissues and, in turn, decrease the suitability of host plants to aphids and leafminers (Facknath & Lalljee, 2005; Myers & Gratton, 2006). Preliminary tests at our laboratory revealed that the total potassium (dry weight) accumulated in hydroponically grown cotton can be increased from ≈3.0 to ≈8.0% KCl by simply adding 3% KCl directly into the standard hydroponic fertilizer solution. The impact of increasing the potassium content in the plant and the subsequent effect it might have on L. hesperus feeding activity is currently under investigation (JR Hagler, unpubl.). Finally, host-plant resistance programs might exploit the deterrent effect potassium has on L. hesperus feeding activity by selecting plants that contain highpotassium concentrations. The total potassium (dry weight) present in many L. hesperus host crops (e.g., cotton, alfalfa) ranges from 1.0 to 5.0% (Jones, 2003). Moreover, potassium concentrations can vary within the same plant species (Waller, 1972; Hagler, 1990). For example, the total potassium present in varieties of cotton can range from 1.1% in Roundup Ready cotton to almost 5% in wild cotton (Percy et al., 2002; JR Hagler, unpubl.). Perhaps natural and induced differences in potassium concentrations across crops and plant species can be exploited for better lygus control.

In summary, our study shows that KCl incorporated into an artificial diet acts as a strong antifeedant for L. hesperus. Further studies have been initiated to determine the feasibility of using KCl as a L. hesperus management tool.

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